

Title: Regulatory Control over Rapid Development in *Marsilea*

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Abstract:

The endosporic male gametophyte of the water fern *Marsilea vestita* provides a unique opportunity to study mechanisms that control cell fate determination during a burst of rapid development. The gametophyte utilizes nine successive cell division cycles in precise planes within a closed volume to produce seven sterile cells and thirty-two spermatids. There is no cell movement in the gametophyte, so cell position and size within the spore wall defines cell fate. After the division cycles are completed, the spermatids are sites for the *de novo* formation of basal bodies, the assembly of a complex cytoskeleton, for nuclear and cell elongation, and for ciliogenesis. The spermatids differentiate into multiciliated, corkscrew-shaped gametes that resemble no other cells in the entire plant. The mature gametes are released approximately 11 h after dry microspores are placed into water, and development is controlled post-transcriptionally. Transcripts stored in the microspore are released (unmasked) in the gametophyte at different times during development. At the start of these studies, we identified several key mRNAs that undergo translation at specific stages of gametophyte development. We developed RNA silencing protocols that enabled us to block the translation of these proteins and thereby established their necessity and sufficiency for the completion of specific stages of gametogenesis. In addition, RNAi enabled us to identify additional proteins that are essential for other phases of development. Since the distributions of mRNAs and the proteins they encode are not identical in the gametophyte, it is clear that transcript processing is important in allowing translation to occur under strict temporal and spatial control. Transcript polyadenylation occurs in the spermatogenous cells in ways that match the translation of specific mRNAs. We have found that the exon junction complex (EJC) plays key roles in transcript processing that underlie cell specification in the gametophyte. We have recently become interested in the mechanisms that control the unmasking of the stored transcripts, and have linked the synthesis and redistribution of spermidine in the gametophyte to the control of mRNA release from storage during early development, and later to basal body formation, cytoskeletal assembly and to nuclear and cell elongation in the differentiating spermatids.